Role of Apaf-1 in Melanoma Drug Resistance and Apoptosis

To the Editor:

The inability to undergo apoptosis in response to chemotherapy and other external stimuli poses a selective advantage for tumor progression, metastasis formation as well as resistance to therapy in melanoma (Soengas and Lowe, 2003). Recently, Apaf-1, known to be critically involved in the mitochondrial apoptotic pathway, was described as being frequently downregulated in melanoma primary and metastatic cell lines on protein and RNA by Soengas *et al* (2001), which was suggested as a therapeutic tool in melanoma treatment. A negative correlation of Apaf-1 to malignancy, drug resistance, and tumor progression in melanoma and other tumors has also been shown by other groups (Jia *et al*, 2001; Fu *et al*, 2003; Baldi *et al*, 2004). In addition, a higher frequency of allelic imbalance of the Apaf-1 locus in metastatic compared with primary melanoma was discovered (Watanabe *et al*, 2003; Fujimoto *et al*, 2004). In contrast to these studies, our experiments using the drug-sensitive parental melanoma cell line MeWo, and its various resistant sublines show that Apaf-1 expression is not related to apoptosis deficiency and drug resistance.

We have analyzed Apaf-1 expression in relation to chemoresistance and apoptosis upon drug treatment in a well-characterized melanoma cell system (Kern *et al*, 1997; Lage *et al*, 1999; Helmbach *et al*, 2002; Wittig *et al*, 2002) of drug-sensitive and -resistant variants, all generated from a parental melanoma cell line by continuous drug exposure to cisplatin ($MeWo_{Cis1}$), etoposide ($MeWo_{Eto1}$), fotemustine ($MeWo_{Fote40}$), and vindesine ($MeWo_{Vin5}$) (Kern *et al*, 1997).

In all MeWo cells, no relevant mutation of p53 was detected (data not shown). Drug-resistant variants differ with respect to DNA repair (Lage *et al*, 1999) and differential apoptotic response toward drug exposure (Helmbach *et al*, 2002). Apoptotic features have been analyzed at a corresponding cell survival rate (25% [IC_{75}] and 5% [IC_{95}]) upon drug treatment. In contrast to sensitive cells, a marked apoptosis deficiency was observed in etoposide-resistant cells including characteristics of apoptotic cell death such as DNA fragmentation (Fig 1*B*, *lanes 3* and *4*), PARP cleavage, and effector-caspase activation. In addition, cytochrome *c* release was strongly reduced and caspase-9 activation was not found compared with sensitive cells (Helmbach *et al*, 2002). Despite the significant apoptosis deficiency observed, there was no differential Apaf-1 expression on the protein level compared with sensitive cells (Fig 1*A*, *lane 3*). Although cisplatin-resistant cells expressed significantly higher levels of Apaf-1 than sensitive cells (Fig 1*A*, *lane 2*), caspase-9 activation as well as effector-caspase activation were slightly reduced (Helmbach *et al*, 2002). Furthermore, the final DNA fragmentation was not significantly altered in cisplatin-resistant cells (Fig 1*B*, *lanes 1* and *2*). Furthermore, in fotemustine-resistant cells, where no differential expression of Apaf-1 was observed (Fig 1*A*, *lane 4*), DNA fragmentation upon fotemustin exposure was markedly increased (Fig 1*B*, *lanes 5* and *6*). Vindesine-resistant cells exhibited a strong upregulation of Apaf-1 on

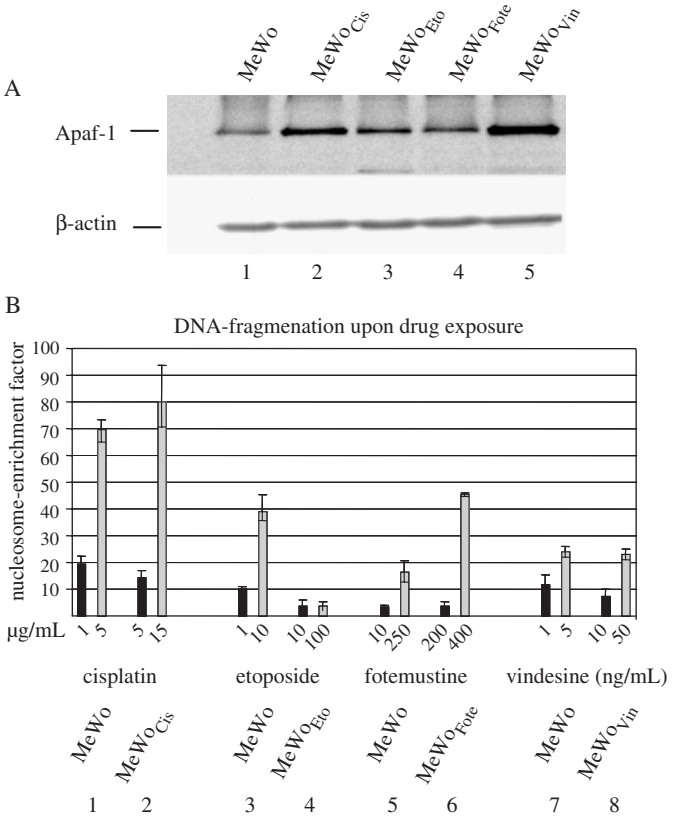

Figure 1
(*A*) Western blot analysis of Apaf-1 in whole-cell lysate of drug-sensitive melanoma MeWo cells (*lane 1*) and their drug-resistant sublines: cisplatin resistant (*lane 2*), etoposide resistant (*lane 3*), fotemustin resistant (*lane 4*), and vindesin resistant (*lane 5*). The monoclonal mouse anti-Apaf-1 antibody was kindly provided by Yuri Lazebnik. Equal amounts of protein were confirmed by detection of β-actin. (*B*) DNA fragmentation is induced at corresponding survival rates, 25% [IC_{75}] (*black bar*) and 5% [IC_{95}] (*gray bar*) in sensitive and resistant cells upon drug treatment using histone-associated DNA fragments (mono- and oligonucleosomes) released from nuclear fractions of drug-treated cells that were quantified using a Cell Death Detection ELISA (Roche, Penzberg, Germany) according to the manufacturer's instructions. Cells were treated for 72 h. The respective drug concentrations are shown below the bars. Data were normalized against the control and interpreted as the nucleosome enrichment factor. Standard deviations were below 8% in all sets of experiments.

protein level (Fig 1*A*, *lane 5*). But no apoptosis deficiency, associated with DNA fragmentation, could be observed compared with sensitive cells (Fig 1*B*, *lanes 7* and *8*).

Taken together, these observations demonstrate that in this melanoma cell system of drug-sensitive and -resistant variants, Apaf-1 expression does not allow to predict chemosensitivity, as was suggested by Soengas *et al* (2001) and Baldi *et al* (2004). Furthermore, apoptosis deficiency is not associated with reduction of Apaf-1. In contrast, there are two drug-resistant sublines ($MeWo_{Cis}$ and $MeWo_{Vin}$) that show a strong upregulation of Apaf-1 on the protein level compared with sensitive cells. Both lines showed no significant increase in DNA fragmentation upon cisplatin or vindesine treatment compared with sensitive cells. Furthermore, two drug-resistant lines without significant differential Apaf-1 expression demonstrated different apoptotic activity upon drug treatment. Whereas etoposide-resistant cells present a marked reduction of DNA fragmentation, fotemustine-resistant cells show an increase.

Our data strongly support the results of Zanon *et al* (2004), who investigated Apaf-1 expression in a large panel of human melanoma and assessed cellular response to several proapoptotic agents in tumors expressing or lacking Apaf-1. The authors showed that the response of various human melanoma cells to different apoptotic agents was independent of the Apaf-1 phenotype.

We suggest that specific regulation of the apoptotic cascade is strongly dependent on the cell type and the apoptotic stimulus, and differs between different cytostatic drugs. Because of further apoptosome-independent apoptotic molecules, loss of Apaf-1 might be bypassed and does not necessarily cause apoptosis deficiency. Conversely, an increase of Apaf-1 does not automatically lead to drug sensitivity.

The data presented here are results obtained on cell clones selected *in vitro* from a single parental cell line, and therefore they cannot be generalized. Nevertheless, these results clearly show no significant correlation between Apaf-1 expression in melanoma and malignancy. This is in contrast to several investigations in the recent past that showed reduced Apaf-1 expression with tumor progression. In our melanoma cell system, however, Apaf-1 levels are clearly not predictive of drug sensitivity, which suggests that the earlier findings cannot be generalized either. Finally, the influence of Apaf-1 expression on patient survival still remains as a critical question.

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